

# Cladocera remains and vegetation types to assess the state of oxbows

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## Abstract

We assessed the usefulness of Cladocera remains for establishing the ecological status of oxbows and also tested the association of Cladocera species with various vegetation types. Cladocera remains were collected from the surface sediment of four habitat types (tangled vegetation, open water, reeds and tunnels) and 15 physical and chemical parameters of surface water were studied. In the surface sediment samples, we identified 32 Cladocera taxa. There was a significant difference in the number of species amongst habitat types as per ANOVA. The benthic and plant associated Cladocera communities of reeds, tangled vegetation, open water and tunnels were clearly separated from each other by NMDS ordination. CCA showed that habitat types had characteristic Cladocera species: *Pleuroxus* species were frequent in the tangled vegetation habitat, while *Chydorus* species were frequent in the open water. Remarkably, in reeds, *Bosmina* species were frequent, although these species are usually common in open water. Specimens of the *Alona* genus were found everywhere. Our findings suggest that the remains of Cladocera species may be useful indicators to assess and monitor the structure of freshwater lakes.

## Keywords

Water-Sediment chemistry, Macrophytes, Zooplankton Indicator species



## Introduction

There are several organic and inorganic remains in sediments which reflect the history of oxbows. In lake sediments, some of the most common animal remains are those of Cladocera which derive from both water and sediment (Kurek et al. 2010). The taxonomic structure of Cladocera remaining in sediment cores indicates past changes in the environment, such as eutrophication (Visconti et al. 2008, Nevalainen and Luoto 2013), acidification (Jeziorski et al. 2008) and changes in the water level (Korponai et al. 2016). Cladocera communities play an important role in a lake's food web because of their intermediate position, which means that Cladocera species have significant effects on the ecology status and water quality of lakes (Jeppesen et al. 2011, Zhi et al. 2012). Due to this position in the food web, Cladocera are sensitive indicators of environmental changes (Kurek et al. 2010).

Earlier studies indicated that the boundary zone between macrophyte beds and open water is particularly important as a refuge for cladocerans (Lauridsen et al. 1996; Davidson et al. 2010). At the same time, Cladocera communities vary with macrophyte bed size and open water which is an important daytime refuge for potentially migrating pelagic cladocerans (Lauridsen et al. 1996).

In this study, we tested the effect of habitat types of oxbows on Cladocera communities in the Upper Tisza Region, in northeast Hungary. We also studied the correlation between the water chemical parameters of Cladocera species. We hypothesised that there were Cladocera species characteristic of typical oxbow habitats and that they are useful indicators for assessing and monitoring the structure and ecological state of lakes.

## Methods

### Study sites

Many oxbows were formed during the 19th century with the controlling of the River Tisza. To assure shipping and flood-control, more than 100 meanders were cut. As a result, many artificial oxbow lakes were formed along the River Tisza (Babka et al. 2011, Balogh et al. 2016, 2017, Kunderát et al. 2017). In the Upper-Tisza region, there are more than 40 oxbows. This region is mainly cultivated using traditional agricultural systems with meadows, orchards and some cereal cultivation. The characteristic land use of this countryside has changed considerably during the last 200 years (Varga et al. 2013).

The oxbows studied were in the Upper Tisza region, near the town of Vásárosnamény in Hungary. The following oxbows were studied: Keskeny Holt-Tisza (48°9'5.64"N, 22°20'9.30"E), Foltos-kerti Holt-Tisza (48°5'47.58"N, 22°23'47.64"E) and Patkó Holt-Tisza (48°6'27.66"N, 22°23'1.56"E). In each oxbow, four sampling points were chosen to represent typical habitat types: tangled vegetation, open water, reeds and



tunnels. In the tangled habitat, *Ceratophyllum demersum* (about 90%) and *Potamogeton natans* (about 10%) were the most abundant plant species. In the open water habitats, there were no aquatic plants. In the reed habitat, the main aquatic plant species was *Phragmites australis*. The tunnel habitats were at least 1.2 m deep and wide open surfaces with little vegetation towards the sides were typical (*Ceratophyllum demersum*).

### Cladocera identification

For Cladocera identification, surface sediment subsamples (1 cm<sup>3</sup>) were treated with 100 ml 10% KOH (Normapur, VWR) solution and heated at 100 °C for about 1 hour. Hydrofluoric acid (HF) (38%, VWR) was used to remove the inorganic material following identification. Then we added safranin O (ALFA (AESAR)) to the sample to stain the remains. We prepared quantitative slides by pipetting 100 µl of each subsample on to a microscope slide and then examined it under a microscope (B-183, OPTIKA Microscopes, Italy) at magnifications of 100 and 400; about 200 Cladocera remains were counted from each sample. Usually two slides are sufficient for identifying at least 200 remains, which is the recommended number for counting (Kurek et al. 2010). The identification was based on Bledzki and Rybak (2016) and Szeroczyńska and Sarmaja-Korjonen (2007).

### Water analyses

Surface water samples were collected in plastic bottles and parallel measurements were performed at the study sites. Water depth, transparency, temperature, conductivity (WTW cond. 340i) and pH (WTW pH 315i) were measured. Samples were stored at 4 °C until the laboratory process. In the laboratory, the content of suspended solids, chlorophyll-a, Chemical Oxygen Demand (COD) and the concentrations of carbon dioxide, ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen and orthophosphate were measured. Laboratory analyses of water samples were based on APHA (2000) and Nollet and De Gelder (2011).

### Sediment analyses

To determinate the organic matter content of surface sediment, the loss on ignition method was used. After drying at 105 °C, 0.2 g samples were cremated at 550 °C for 4 h in a muffle furnace (Nabertherm L5/C6, Germany). The loss on ignition was calculated with the following equation:  $LOI_{550} = 100 \cdot (DW_{105} - DW_{550}) / WS$ , where  $LOI_{550}$  was the percentage loss on ignition at 550 °C,  $DW_{105}$  was the dry weight of samples at 150 °C and  $DW_{550}$  was the weight of the sample at 550 °C (Heiri et al. 2001, Matthews 2014). To determine the content of calcium carbonate in the surface



sediment, the samples were burnt at 950 °C for 4 h. After cooling, we measured them with analytic scales. The calculation of the loss on ignition was conducted with the following equipment:  $LOI_{950} = 100 \cdot (DW_{550} - DW_{950}) / WS$ , where  $LOI_{950}$  is the percentage of loss on ignition at 950 °C and  $DW_{950}$  is the weight of the sample after heating at 950 °C (Heiri et al. 2001, Matthews 2014).

## Statistical analyses

The benthic and plant associated Cladocera communities were studied, based on vegetation types, by non-metric multidimensional scaling (NMDS) ordination. CCA was used to display the correlation between water chemistry and the Cladocera community (Lepš and Šmilauer 2003). One-way ANOVA was used to test the effect of habitat types on Cladocera diversity and water chemistry. In the case of significant differences, Tukey's Multiple Comparison test was used (Abbott 2016).

## Results

### Cladocera diversity

In total, we counted 1324 Cladocera specimens in the samples; altogether, we identified 32 taxa (Table 1). There was a significant difference in the number of Cladocera species amongst the vegetation types by ANOVA ( $F_{3,8} = 4.744$ ,  $P = 0.034$ ) (Fig. 1). A significantly higher number of Cladocera species was found in the open water than in the reed vegetation type ( $P < 0.05$ ). There was no significant difference in the number of Cladocera individuals amongst the vegetation types (oxbows:  $F_{3,8} = 0.500$ ,  $P = 0.693$  Fig. 2).

The benthic and plant associated Cladocera communities of reeds, tangled vegetation, open water and tunnels were clearly separated from each other by NMDS ordination. The communities of benthic Cladocera in tangled vegetation, open water and tunnels were similar to each other (Fig. 3). A similar result was found in the cases of plant associated Cladocera communities when using NMDS ordination (Fig. 4).

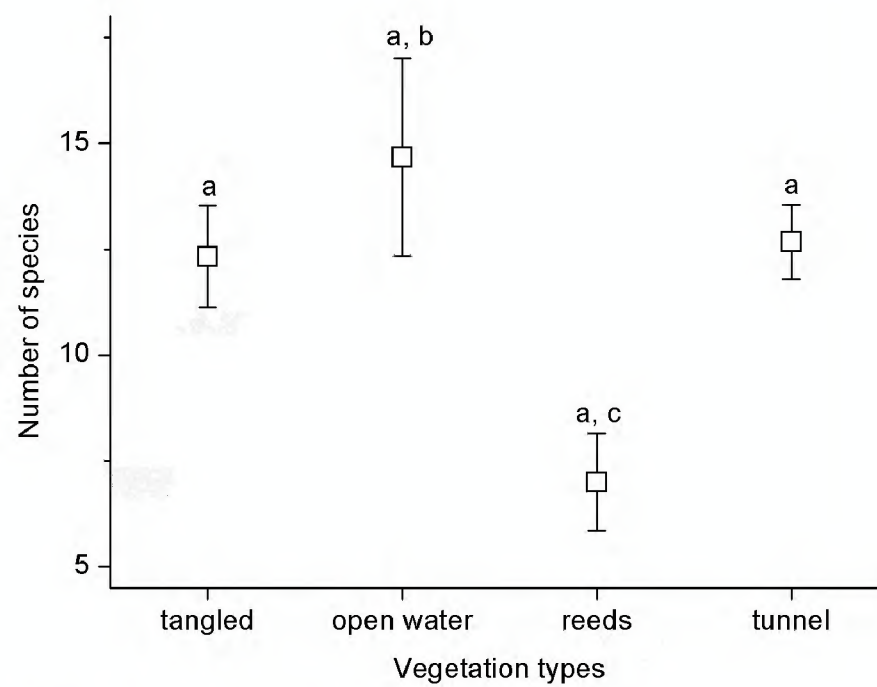
### Water physico-chemistry and sediment chemistry differences amongst vegetation types

There were no significant differences in the water physico-chemistry parameters studied (depth:  $F = 1.234$ ,  $p = 0.359$ ; visibility:  $F = 0.591$ ,  $P = 0.638$ ; temperature:  $F = 0.164$ ,  $P = 0.918$ ; pH:  $F = 2.433$ ,  $P = 0.140$ ; conductivity:  $F = 0.029$ ,  $P = 0.993$ ; suspended solids:  $F = 1.038$ ,  $P = 0.427$ ;  $CO_2$ :  $F = 2.519$ ,  $P = 0.132$ ; COD:  $F = 0.004$ ,  $P = 1.000$ ;  $NH_4^+$ :  $F = 1.406$ ,  $P = 0.310$ ;  $NO_3^-$ :  $F = 0.696$ ,  $P = 0.580$ ; Chlorophyll-a:  $F$

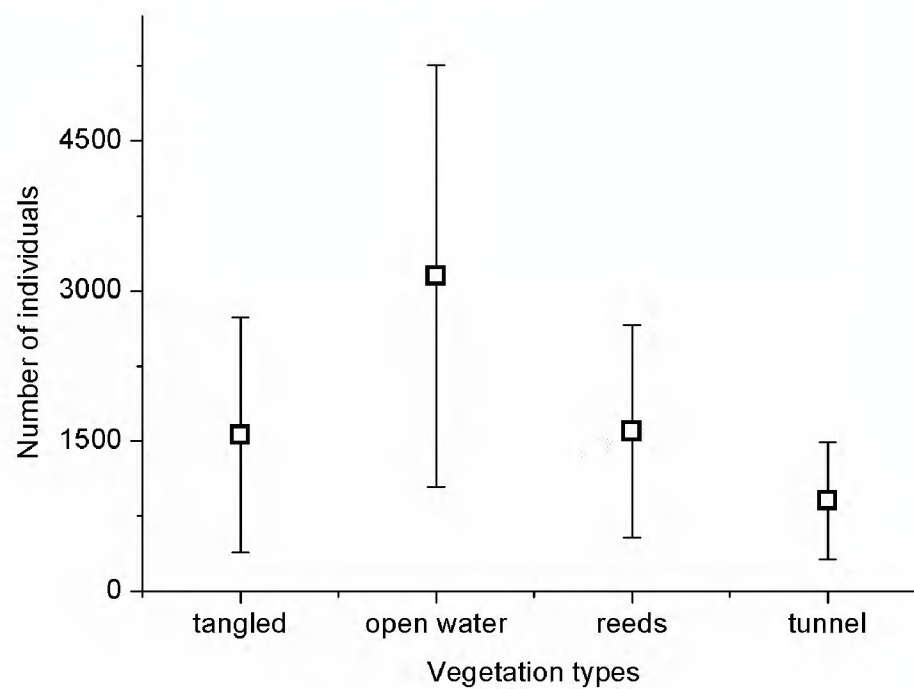


**Table 1.** Summary of Cladocera species and individual numbers based on the oxbows and vegetation types studied.

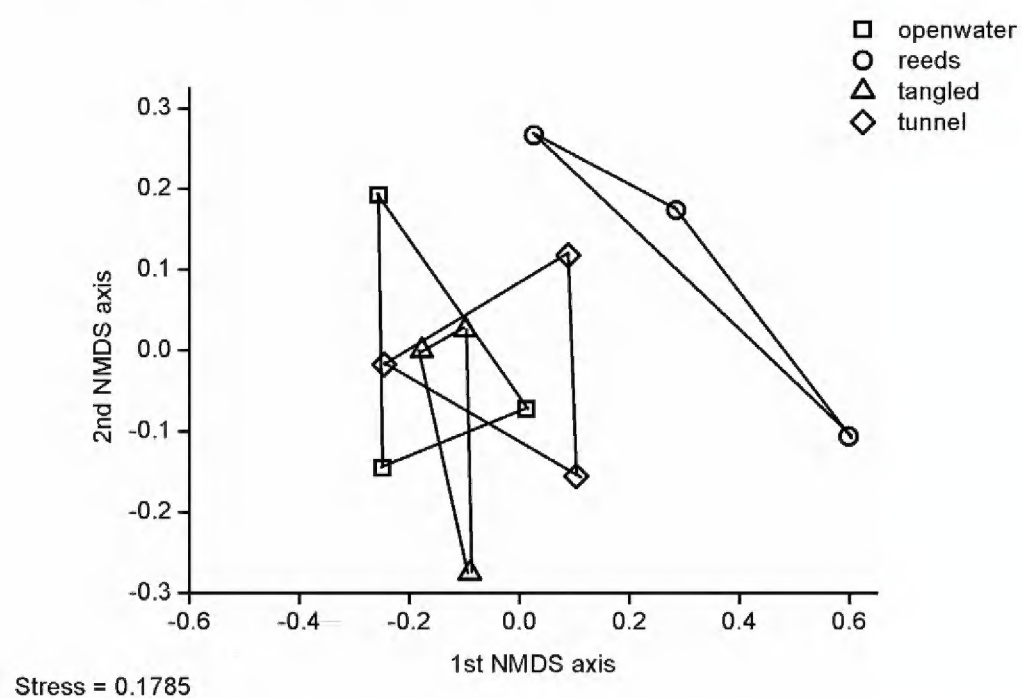
	Habitat affinity	Keskeny Holt-Tisza				Foltos-kerti Holt-Tisza				Patkó Holt-Tisza			
		tangled veg- etationn	open	reeds	tunnel	tangled veg- etationn	open	reeds	tunnel	tangled veg- etationn	open	reeds	tunnel
<i>A. affinis</i>	reeds	125	50	0	0	10	7	17	0	3	3	8	13
<i>A. elongatus</i>	sediment	25	0	0	0	0	0	0	0	0	0	0	0
<i>A. emarginatus</i>	tangled vegetation	0	0	0	0	0	0	33	0	0	0	0	0
<i>A. excisa</i>	tangled vegetation/ reeds	0	0	0	50	5	0	0	0	0	18	2	0
<i>A. exigua</i>	tangled vegetation	75	0	0	0	5	0	67	0	3	0	0	0
<i>A. guttata</i>	tangled vegetation/ reeds	150	50	0	17	10	0	67	0	40	4	0	0
<i>A. harpae</i>	plants	100	0	0	0	0	7	33	0	0	9	0	0
<i>A. intermedia</i>	sediment	0	250	25	17	10	7	83	22	48	7	0	25
<i>A. nana</i>	plants	25	50	0	17	0	0	0	0	0	0	0	13
<i>A. quadrangularis</i>	sediment/plant	75	100	25	0	0	0	0	67	5	0	0	41
<i>A. rectangula</i>	sediment	225	400	0	0	35	54	183	0	113	3	0	16
<i>B. coregoni</i>	open water	650	1050	1050	900	40	39	167	700	0	3	2	44
<i>B. longirostris</i>	plants/open water	2075	4600	2350	683	300	196	1133	344	0	1	2	53
<i>B. longispina</i>	open water	0	150	75	133	5	0	67	0	0	0	0	0
<i>C. fennicus</i>	sediment	0	0	0	0	0	4	17	0	0	0	0	0
<i>C. gibbus</i>	sediment	0	0	0	0	0	0	17	0	0	0	0	0
<i>C. rectirostris</i>	plants	0	0	25	33	0	0	0	0	0	0	0	6
<i>C. sphaericus</i>	sediment	175	200	25	50	25	18	150	11	25	53	2	38
<i>D. longispina</i>	open water	0	0	0	0	60	0	0	0	0	0	0	13
<i>D. rostrata</i>	sediment	25	50	0	50	0	0	17	0	0	0	0	0
<i>E. lamellatus</i>	sediment/plant	0	0	25	0	0	0	0	0	0	0	0	0
<i>G. testudinaria</i>	plants	150	150	0	0	10	7	17	0	0	0	0	0
<i>K. latissima</i>	plants	0	0	0	0	0	0	0	0	0	3	0	0
<i>L. acanthocercoides</i>	sediment/plant	0	0	0	0	0	0	0	0	0	0	0	6
<i>L. leydigi</i>	sediment	0	50	25	33	0	0	0	0	0	0	2	6
<i>M. dispar</i>	sediment	0	0	0	0	0	0	0	0	0	0	2	0
<i>O. tenuicaudis</i>	tangled vegetation/ reeds	0	0	0	0	0	0	17	0	10	0	0	0
<i>P. laevis</i>	plants	0	0	0	50	0	7	17	0	0	0	0	9
<i>P. trigonellus</i>	sediment/plant	25	50	0	17	10	11	33	0	3	0	0	0
<i>P. truncatus</i>	tangled vegetation/ reeds	0	0	0	0	0	0	17	0	13	0	0	0
<i>P. uncinatus</i>	sediment	0	0	0	0	0	0	0	0	0	1	0	0
<i>S. crystallina</i>	plants/open water	0	0	0	17	0	0	0	0	0	0	0	0



**Figure 1.** Number of Cladocera species by vegetation type.

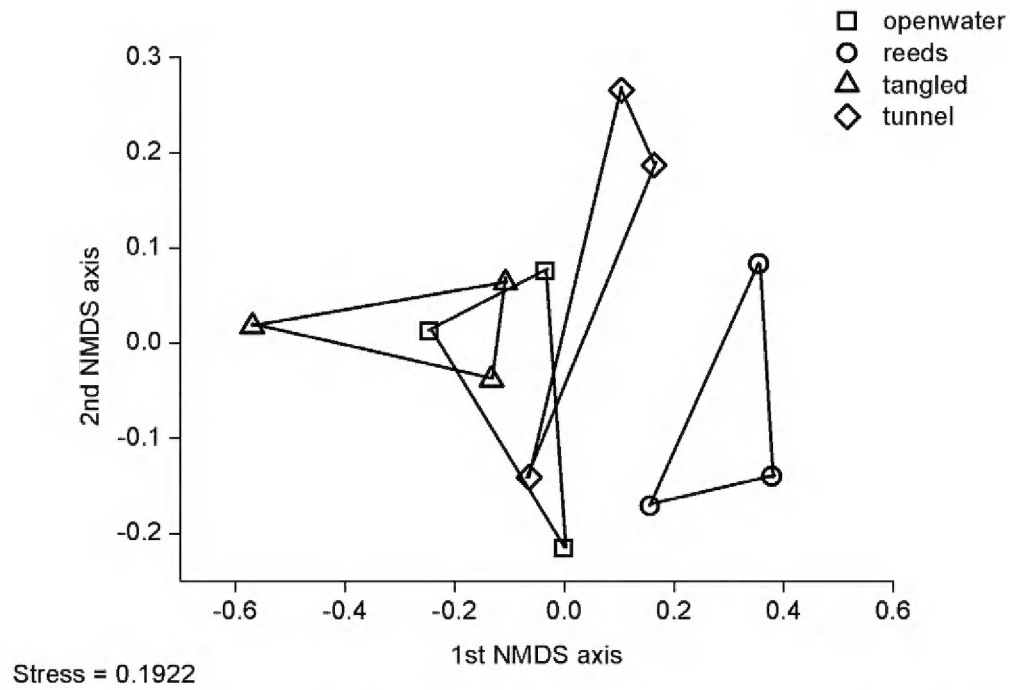


**Figure 2.** Number of Cladocera individuals by vegetation type.



**Figure 3.** NMDS ordination of the benthic Cladocera remains of vegetation types (stress=0.1785).





**Figure 4.** NMDS ordination of the plant associated Cladocera remains of vegetation types (stress=0.1922).

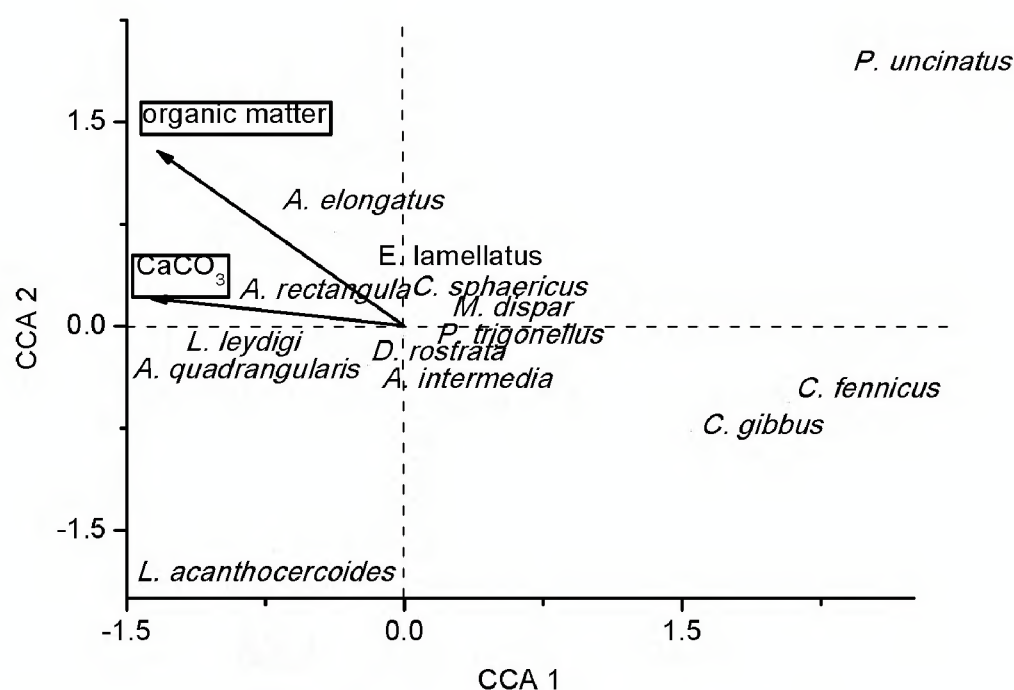
= 2.823,  $P = 0.107$ ) amongst vegetation types (Table 2). Similar to the water physico-chemical parameters, significant differences were not found amongst vegetation types based on the organic matter content ( $F = 3.159$ ,  $P = 0.086$ ), nor on the calcium carbonate content ( $F = 0.134$ ,  $P = 0.937$ ) of sediment (Table 2).

### Correlation between sediment and water chemistry and Cladocera communities

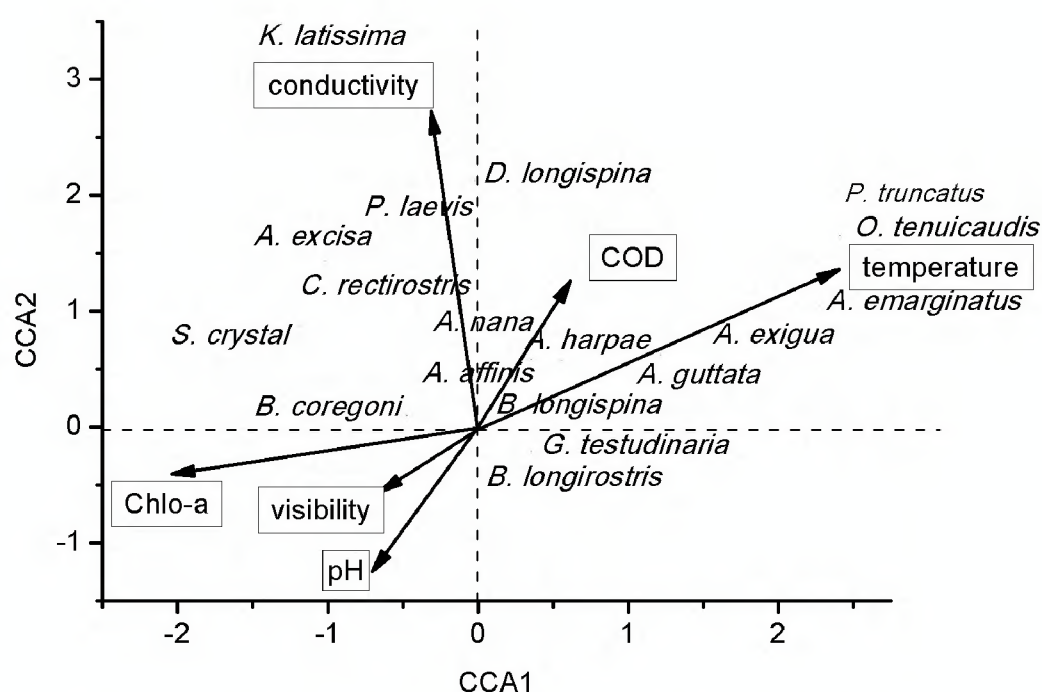
Based on the CCA ordination, our results show positive correlations between the organic matter and the calcium carbonate content of sediment and *A. elongatus*, *A. rectangular*, *L. leydigi* and *A. quadrangularis* species (Fig. 5). In the cases of water chem-

**Table 2.** Physical and chemical parameters of surface water and sediment (mean  $\pm$  SD) according to vegetation type.

Parameters	Vegetation type			
	tangled vegetation	open water	reeds	tunnel
<b>Water</b>				
depth, cm	59 $\pm$ 20	80 $\pm$ 20	135 $\pm$ 35	75 $\pm$ 20
visibility, cm	49 $\pm$ 21	54 $\pm$ 7	73 $\pm$ 23	47 $\pm$ 4
temperature, °C	12 $\pm$ 1	11 $\pm$ 1	11 $\pm$ 1	11 $\pm$ 1
pH	8.3 $\pm$ 0.3	8.6 $\pm$ 0.2	8.5 $\pm$ 0.3	7.9 $\pm$ 0.1
conductivity, $\mu\text{S cm}^{-1}$	334 $\pm$ 78	346 $\pm$ 73	343 $\pm$ 73	370 $\pm$ 920
suspended solid, $\text{mg l}^{-1}$	8 $\pm$ 4	10 $\pm$ 2	12 $\pm$ 8	3 $\pm$ 1
$\text{CO}_2$ , $\text{mg l}^{-1}$	19 $\pm$ 5	10 $\pm$ 2	24 $\pm$ 3	15 $\pm$ 4
COD, $\text{mg l}^{-1}$	6 $\pm$ 5	4 $\pm$ 2	5 $\pm$ 3	6 $\pm$ 4
$\text{NH}_4^+$ , $\text{mg l}^{-1}$	3 $\pm$ 1	1 $\pm$ 1	2 $\pm$ 1	1 $\pm$ 1
$\text{NO}_3^-$ , $\text{mg l}^{-1}$	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1
Chlorophyll-a, $\text{mg l}^{-1}$	6 $\pm$ 2	9 $\pm$ 3	15 $\pm$ 7	4 $\pm$ 1
<b>Sediment</b>				
organic matter, %	4.1 $\pm$ 0.4	3.4 $\pm$ 0.2	2.4 $\pm$ 1.0	2.9 $\pm$ 0.8
$\text{CaCO}_3$ , %	0.7 $\pm$ 0.2	0.8 $\pm$ 0.3	0.7 $\pm$ 0.5	0.6 $\pm$ 0.2



**Figure 5.** CCA ordination of benthic Cladocera taxa and sediment parameters.



**Figure 6.** CCA ordination of Cladocera taxa and water chemical parameters.

istry, data positive correlations were found between conductivity and *K. latissima* species, between chemical oxygen demand and *A. nana*, *A. harpae* and *A. exigua* species and between temperature and *P. truncatus*, *O. tenuicaudis* and *A. emarginatus* (Fig. 6).

## Discussion

Our study demonstrated the usefulness of Cladocera remains in the assessment of the ecological status of oxbows. Similarly to our study, Gulyás and Forró (1999) and Korhola and Rautio (2001) also demonstrated a correlation between habitat types and Cladocera species. The CCA results corroborated the habitat preferences reported by Gulyás and Forró (1999) and Korhola and Rautio (2001). At the tunnel of the Kes-



keny Holt-Tisza oxbow, we found the kind of Cladocera species which usually live in vegetation zones. In the tangled vegetation of the Keskeny Holt-Tisza oxbow, we found *Alonella exigua*, *Acroperus harpae* and *Alona guttata* which were typical tangled vegetation species; however, *Acroperus elongatus* and *Alona affinis* were mostly living in the biotecton of vegetation. In the tangled vegetation of the Foltos-kerti Holt-Tisza, *Alonella excisa* was a typical tangled vegetation species. We also found *Daphnia longispina* remains there; this species is characteristically an open water and/or generalist species. In tunnels, *Alona quadrangularis* was a benthic species as reported by literature. The Cladocera species we found in the tangled vegetation of the Patkó Holt-Tisza oxbow usually lived in tangled vegetation and in sediment. In the open water, we found the kind of species which usually live in sediment and in vegetation and not usually in open water. Probably these species are able to adapt quickly to the modified environmental conditions caused by human disturbance (i.e. the intense utilisation of the oxbow for recreational fishing).

There were significant differences amongst oxbows and the habitat types based on water chemistry parameters. Similar to earlier studies (Lukács et al. 2009, 2011), we found that aquatic plants influenced the water chemistry parameters. Lukács et al. [(2011) demonstrated that the amount of chlorophyll-a was very high in sweet grass beds communities, but a small amount of chlorophyll-a was found in chestnut and water lily beds. Our findings also demonstrated that there was a strong interaction between water chemistry parameters and reed habitats.

We found that temperature was in a positive correlation with the number of Cladocera individuals. Nevalainen and Luoto (2010) also reported that many Cladocera species are sensitive to seasonal temperature changes. Zawisza et al. (2016) and Wojewódka et al. (2016) reported that several studies described strong correlations between pH, conductivity and Cladocera taxa. Bjerring et al. (2009) found negative correlations between temperature and chlorophyll-a and several Cladocera taxa. We found a positive correlation between conductivity and Cladocera taxa, while a negative correlation was found between pH and Cladocera taxa.

## Conclusions

Our results show that Cladocera taxa are usually associated with characteristic habitat types; however, human disturbance can change the habitat association of these species by changing the local environment conditions. Based on our results, Cladocera are useful indicators for assessing and monitoring the structure of freshwater lakes.

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